NOTES ON FIBRIN, OXYHÆMOGLOBIN CRYSTALS, AND THE COLLODION METHOD.

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I.

Preparation of the Fibrin Filaments or Net-Work of Blood and Lymph.

The ordinary methods of demonstrating microscopically the minute net-work or filaments of fibrin formed in the coagulation of blood or lymph are so unsatisfactory that students in histology are practically debarred from the observation at once so instructive and interesting. The following note is the result of experiments carried on during the last two years, in which many classes of vertebrates have been studied and one invertebrate—the cray-fish. The method finally adopted is very simple and the results are all that could be desired, so that it is as easy a demonstration as most of those made on the blood by beginning students. The main features of the method were presented at the Meeting of the American Association of Anatomists, held in Boston, 1890, and abstracted in the report of the association, 1891, pp. 25–26. The method is briefly as follows: A drop of perfectly fresh, uncoagulated blood or lymph is placed on a slide or cover-glass and the cover is then put on a slide and pressed down moderately, so that there will not be too thick a layer. The cover is also made to project slightly over the edge, as shown in Fig. 1 C, so that it may be easily grasped and raised; or, the method that has proved more satisfactory in the laboratory with students, the blood or lymph is put on a cover-glass and another cover is put on it. The two are pressed quite firmly together and made somewhat eccentric, so that the projecting edge may be grasped more easily, as in the case where a slide is used. As soon as the preparations are made they are put on the glass bench in the moist chamber to prevent drying (Fig. 1 A). In the course of 10 to 30 minutes the coagulation will be complete in most cases. Indeed, coagulation

may be complete within three minutes in some cases. A sojourn of 24 hours in the moist chamber does no harm in most instances.

After the coagulation is complete, if one desires to preserve the blood or lymph corpuscles a drop of normal salt solution is drawn around the edge of the cover-glass, or if only the fibrin net-work is desired, then water is used. This softens the blood around the edge of the cover. Finally, a needle may be used to remove any partly dried blood on the edge. This is unnecessary in most cases, however. The cover is then grasped with forceps, or a needle is used, and the cover raised. The fibrin net-work nearly always adheres to the cover-glass if a slide and cover are used. When two covers are used it is immaterial which one retains the fibrin. It is especially desirable to avoid sliding the cover when raising it, for by sliding it the fibrin net-work is very liable to be thrown into folds.

When the cover is removed, the fibrin net-work on it is thoroughly but gently washed with normal salt solution or water to remove the soluble proteids and the loose corpuscles. A convenient method of washing is by means of a small pipette like the common medicine droppers. After the preparation is washed it may be stained with aqueous hæmatoxylin 3 to 4 minutes and then with an aqueous solution of eosin one minute. After washing as usual the preparation is set on edge to dry spontaneously. When dry it is mounted over a shallow cell of shellac or other good cement. It is especially necessary that the preparation should be thoroughly dry before mounting. A gentle heat, such as exists at considerable distance above an alcohol-lamp flame, is excellent for the final drying.

It will be found on examining preparations of blood or lymph fibrin so prepared that the fibers or filaments of the net-work are very much coarser in mammalian blood or lymph than the filaments from the blood or lymph of a cold-blooded animal like a frog or Necturus. Indeed, in making the preparations of the cold-blooded animals it is necessary to work with the greatest rapidity, as coagulation takes place much more rapidly than in mammals, although the contrary is sometimes stated in works on physiology (*e. g.*, Landois, p. 54).

Exceedingly thin layers of blood may be obtained by wiping the blood away from the edge of the cover when it is pressed down, so that it will not be drawn back under the cover by capillarity. In most cases the preparations are more satisfactory if the net-work is not too attenuated.

For the satisfactory study of the fibrin net-work of the Batrachia

(frog, etc.) high powers and excellently defining objectives will be found necessary.

For photographing, a stain of an aqueous or an alcoholic solution of picric acid has been found quite satisfactory. The preparation should be well washed if picric acid is used, or minute crystals of picric acid will appear and mar or destroy the preparation after it has stood some time. Eosin-stained specimens also photograph well.

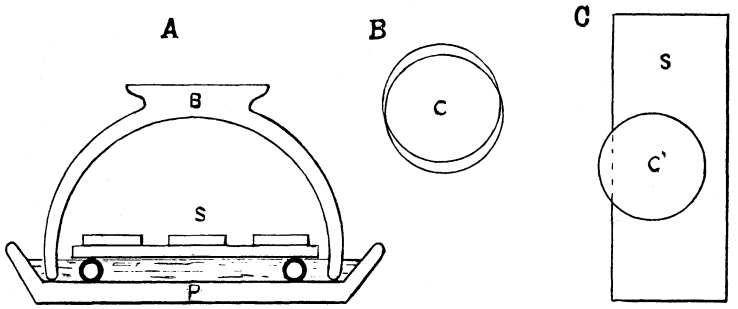

FIG. 1.—Apparatus for the Preparation of the Fibrin Filaments or Network of Blood and Lymph.

A—Simple form of moist chamber made with a plate and bowl. B, bowl serving as a bell-jar; P, plate containing the water and over which the bowl is inverted; S, slides on which are mounted preparations which are to be kept moist. These slides are seen endwise and rest upon a bench made by cementing short pieces of large glass tubing to a strip of glass of the desired length and width.

B—Two cover-glasses (C) made eccentric, so that they may be more easily separated by grasping the projecting edge.

C—Slide (S) with projecting cover-glass (C). The projection of the cover enables one to grasp and raise it without danger of moving it on the slide and thus folding the fibrin net-work.

II.

Preparation of Large Oxyhæmoglobin Crystals from the Blood of Necturus.

The oxyhæmoglobin of Necturus blood very readily crystallizes. As pointed out by Richardson long ago (Trans. Amer. Med. Assoc., 1870), it may crystallize inside the red corpuscles. If blood is defibrinated and ether or chloroform added to the blood to dissolve the hæmoglobin, crystals in great multitudes will be formed as the ether, etc., evaporate. These crystals are perfect in form, but ex-

ceedingly small. They may be quite satisfactorily preserved by drying in a thin layer on a slide or cover-glass. In order, however, to get large, perfect crystals the following method will succeed: A drop of fresh blood, preferably after defibrination, is placed on a slide and an equal volume of 2 per cent. chloral hydrate in water added. The two are well mixed and covered and the cover sealed with castor oil to prevent evaporation. In a day or two crystals of great size and perfection are to be found in the preparation. The crystals are so large that there is no difficulty in getting one to fill the slit of the micro-spectroscope and to show the characteristic absorption sprectrum of oxyhæmoglobin.

No way has yet been devised for rendering these large crystals permanent.

III.

Notes on Albumenizing the Slide for the More Certain Fixation of Serial Collodion Sections.

In making series of sections by the collodion method it frequently happens that one or more of the sections become displaced or totally lost. This frequently occurs when the collodion seemed to melt down on the slide perfectly on the application of the dry ether vapor, as described in the proceedings of last year (pp. 126–130), but originally devised by Prof. H. E. Summers. (See Amer. Mon. Micr. Jour., 1887, p. 73. The Microscope, 1887, p. 73.)

The displacement is especially liable to occur with students, who naturally lack in manipulative skill. In order to avoid the difficulty, the plan formerly largely used by photographers with the wet or collodion photography was adopted, viz., the glass slides were coated with a very thin solution of egg-albumen (white of egg, 1 cubic centimeter; water, 200 cc.). The carefully cleaned slides are dipped or soaked in this, and then stood endwise to drain on some blotting paper. The albumenizing may be done very quickly and the slides preserved indefinitely. The coating of albumen adheres to the glass with the greatest tenacity and never shows the slightest trace of coloration with any stain employed. The collodion adheres to this albumen and no trouble was found in making all the sections of a series remain fast, even by the most inexperienced student. Only the grossest carelessness results in failure. For the use of the students there was added to the albumen as given above 2 cc. of glacial acetic acid to prevent decomposition. The acid albumen works equally well with that not acidulated.

Without doubt the cause of failure, with the clove-oil collodion method of sticking paraffine sections to the slide, is due to the fact that the collodion does not adhere firmly enough to the slide. The albumen will adhere to the slide and the collodion adheres with great tenacity to the albumen, so that if one prefers to employ clove-oil collodion to Mayer's albumen for a fixative it may be done with greater success by first albumenizing the slide as described above.

IV.

The Use of Supports or Holders that Sink in the Hardening Medium for Collodion-Imbedded Objects.

In the collodion method as ordinarily used the imbedded object is usually hardened in alcohol of about 80 per cent. or in chloroform, and the object is mounted on a cork. Last year a device was figured (pp. 127–130) for holding the corks down and in an upright position while the collodion was hardening. Doubtless every one has suffered the inconvenience of the great softness of the corks and the discoloration of the alcohol if they remain in it for any length of time. To overcome both these defects various other substances have been used in place of cork. Dr. C. S. Minot called the attention of the writer to the artificially prepared wood fiber known as vulcanite. This answers very well, but has the defect of swelling to a considerable extent in the alcohol. The various forms of hard rubber proved more satisfactory; but the most satisfactory substance on the whole is solid glass rods of the proper size. Colored glass is to be preferred. It is cut into cylinders of the right length and the end made even by coarse emery or a grindstone. The collodion adheres well to the rough end. It may be albumenized if desired, but that has not proved necessary.

The glass holders are sufficiently heavy to sink readily in any liquid that one would be likely to use. It is entirely indifferent to the various liquids also. (See Fig. 2, A and B.)

In case but a few specimens are to be prepared, doubtless the easiest way, if corks are used as holders, is to fasten to the small end of the cork and insert in a homœopathic vial of alcohol or chloroform and invert the vial.

The simplest and most satisfactory way yet employed by the writer for imbedding all collodion-infiltrated objects is to use a small paper box as for paraffin imbedding, vaseline the bottom lightly and imbed by pouring successive layers of thick (*i. e.*, 5 per cent.) collodion

over the object. It is then hardened in chloroform an hour or two and latter in 82 per cent. alcohol. In this way air bubbles are avoided and the mass is tough.

For cutting, the collodion imbedded specimen is removed from the box and either clamped directly in the microtome holder or placed between two pieces of soft cork and then clamped in the holder.

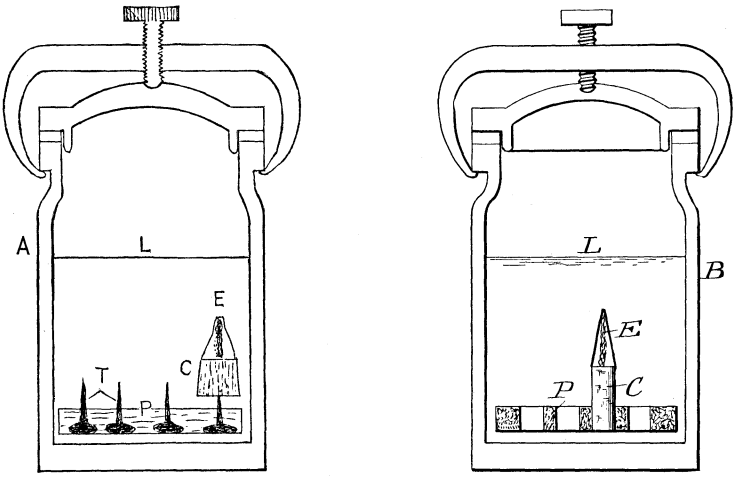

FIG. 2.—Jars for Hardening Collodion-imbedded Objects.

A—From Proceedings of 1890, p. 130. Jar with arrangement for hardening collodion-imbedded objects mounted on corks. P, plaster of Paris disk, in which are imbedded glass tacks (T). The cork (C), on which the specimen (E) is mounted and surrounded by collodion, is pushed down on the glass tacks and thus held under the liquid (L), in which the collodion is hardened.

B—Jar for hardening collodion-imbedded objects mounted on a holder or support heavier than alcohol, the letters as in A, except that C represents the holder made of glass, etc., and thus sinking of its own weight into the holes made in the plaster disk (P).